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Molecular Characterization of β-Thalassemia Genes in an Argentine Population

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This study was designed to identify the β-thalassemia mutations in an Argentine population. Seventy-one pediatric patients and 101 available relatives were studied (85 chromosomes). Diagnosis of β-thalassemia was made by conventional hematological procedures. Molecular studies were carried out by dot-blot and restriction endonuclease analysis on amplified DNA to detect the eight most frequent mutations in the Mediterranean area. We were able to identify 95.3% of the β-thalassemia mutations in the subjects under study. The four common defects (C-39, 47%; IVS-I nt 110, 22.4%; IVS-I nt 1, 9.4%; and IVS-I nt 6, 5.9%) account for 84.7% of the β-thalassemia alleles. The alleles and their distributions showed a close similarity to the spectrum of alleles in Italy. The differences might represent the influence of other immigrations, especially from Spain. We conclude that β-thalassemia in Argentina originated mainly from Italian immigrants. This study will enable us to design an adequate approach to genetic counseling and/or prenatal diagnosis for couples at risk. Am. J. Hematol. 54:179–182, 1997 © 1997 Wiley-Liss, Inc.

Key words: β-thalassemia; molecular biology; mutations; β-globin; Argentina

INTRODUCTION

β-thalassemia (β-Thal) is a genetic disorder characterized by reduced or absent output of β-globin chains. This results in an imbalance of alpha/nonalpha chain synthesis, which is the major determinant of the clinical and hematological severity of the disease. There are two main varieties of β-Thal, i.e., β^0 Thal, in which no β-globin chains are produced, and β^+ Thal, in which some β-globin chains are produced but at a reduced rate [1].

In both heterozygous and homozygous β-Thal, overall alpha/nonalpha chain rate varies widely and depends either on the residual output of β-globin chains from the affected β-globin loci, or on a number of genetic modifying factors [2,3].

β-Thal is extremely heterogeneous at the molecular level. The vast majority, more than 110, are nondeletional forms [4]. Deletions affecting the β-globin gene cluster have also been observed to result in β-Thal phenotype [5]. The world distribution of β-Thal alleles is nonrandom. Each ethnic group carries its own particular repertoire of β-Thal alleles, including a small number of frequent mutations which account for 85–99% of all cases in that population, as well as other, more rare mutations. Thus, in the Mediterranean region more than 30 β-Thal alleles have been described, but only eight point mutations are the most frequent [6].

Knowledge of the frequency and distribution of β-Thal alleles that affects a given ethnic group facilitates molecular detection and also a proper genetic counseling. We characterized the β-globin mutations in 130 individuals (85 chromosomes) with β-Thal in Argentine population in order to facilitate the start of prenatal diagnostic studies.

MATERIALS AND METHODS

Subjects

Between April 1993–December 1995, blood samples from 172 individuals belonging to 66 families with β-Thal were collected. Diagnosis of β-Thal was previously made by conventional hematological analysis in the De-

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partment of Hemato-Oncology, Hospital de Pediatría "Prof. Dr. Juan P. Garrahan," Buenos Aires, Argentina. In 19 families, both parents had β-Thal minor (38 chromosomes), and there was at least one sibling with β-Thal major. Forty-one families have been diagnosed as β-Thal minor (41 chromosomes), and in six families the patients had β-Thal associated with hemoglobin (Hb) S or Hb Lepore (6 chromosomes).

Hematological Analysis

Whole-blood samples were collected with EDTA as anticoagulant. The hematological indices were obtained using an electronic cell counter (Coulter Counter model T-660, Coulter Corporation, Hialeah, Florida). Hemoglobin electrophoresis was performed at pH 8.6 on cellulose acetate [7]. Hemoglobin A_2 was quantitated by the column chromatography procedure [8] (Helena Laboratories, Beaumont, Texas. catalog no. 5342), and Hb F by the alkali denaturation method [9]. Unstable hemoglobins were screened by Isopropanol test [10].

Molecular Analysis

DNA was extracted from peripheral blood mononuclear cells by the standard procedure of cell lysis with proteinase K, Phenol:Chloroform extraction, and Isopropanol precipitation [11]. DNA from each individual studied was used in the polymerase chain reaction (PCR) [12] to detect eight β-Thal alleles. Five segments of the β-globin gene (A–E) were amplified using the primers shown in Figure 1 [13,14]. The point mutations were detected either by restriction enzyme digestion of the respective PCR product followed by gel electrophoresis, or by allele-specific oligonucleotide (ASO) hybridization to the corresponding PCR product dot-blotted onto nylon membranes. Normal and mutant oligonucleotide probes were end-labeled with ^{32}P, using T4 polynucleotide kinase [15].

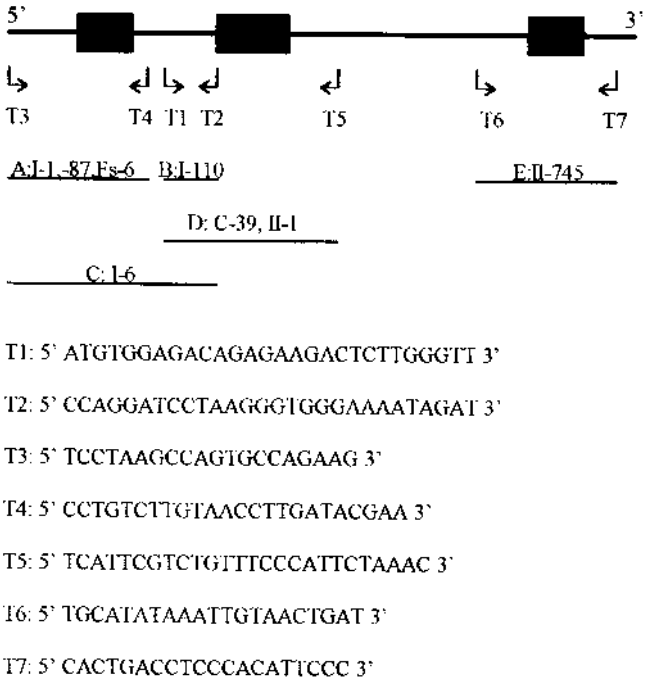

Fig. 1. β-globin gene map and pairs of primers (T1-T2, T3-T4, T1-T5, T2-T3, and T6-T7) used to amplify segments of the gene containing the most frequent β-thalassemia mutations.

TABLE I. Distribution of Alleles Among β-Thalassemia Patients

	Number of		
	Families	Patients	Alleles
β-Thal major	19	22	38
β-Thal minor	41	41	41
β-Thal/Hb S	5	7	4[a]
β-Thal/Hb Lepore	1	1	2
Total	66	71	85

[a]The same allele was shared by one family with β-Thal major and one with β-Thal/Hb S.

RESULTS

One hundred and seventy-two subjects, including 71 pediatric patients and 101 available relatives, were studied between April 1993–December 1995. The corresponding number of chromosomes subjected to analysis was 85. Table I shows the distribution of alleles among β-Thal patients.

The methodology employed was designed to detect the most frequent mutations in the Mediterranean region. Table II shows the frequency and distribution of mutations among different β-Thal patients. The β^0 codon 39 nonsense mutation was the most common defect (47%) followed, in decreasing order, by β^+ IVS-I nt 110 (22.4%), β^0 IVS-I nt 1 (9.4%), β^+ IVS-I nt 6 (5.9%), β^0 IVS-II nt 1 (3.5%), β^+ −87 (2.3%), β^+ IVS-II nt 745 (2.3%), and β^0 Fs-6 (1.2%). Hb Lepore (1.2%) was detected by hematological analysis. We were able to identify 95.3% of β-Thal mutations in the subjects under study. In 4 chromosomes (4.7%), the mutation was not detected by the methodology used. Five of the 22 patients with β-Thal major were genetically homozygous. All the others were compound heterozygous.

The ethnic origins of the subjects studied revealed that 87% of the population had an Italian origin in one or both parents. Two of the 4 unknown chromosomes belong to a Lebanese family. This different racial origin is most likely the reason for their not being detected in our analysis. The geographical distribution of the patients was 80% from Buenos Aires city and surrounding areas, 11% from the Province of Buenos Aires, and 9% from other Provinces.

TABLE II. Distribution and Frequency of Mutations Among β-Thalassemia Patients*

	Nonsense codon 39	IVS-I 110	IVS-I nt 1	IVS-I nt 6	IVS-II nt 1	−87	IVS-II nt 745	Frameshift 6	Hb Lepore	Uncharacterized
β-Thal major	13	11	2	5	1	2	1	1		2
β-Thal minor	24	6	6		2		1			2
β-Thal/Hb S	2	2								
β-Thal/Hb Lepore	1								1	
Total (%)	40 (47)	19 (22.3)	8 (9.4)	5 (5.9)	3 (3.5)	2 (2.3)	2 (2.3)	1 (1.2)	1 (1.2)	4 (4.7)

*β^0 39 (C-T) and β^+ IVSI nt 6 (T-C) were determined by ASO hybridization of duplicated dot-blots containing approximately 50 ng of products D and C, respectively. β^+ IVS-I nt 110 (G-A) and β^0 IVS-I nt 1 (G-A) were analyzed by specific PCR mutagenesis, as described by Lindeman and Hu [13]. β^0 IVS-II nt 1 (G-A) was detected by restriction enzyme digestion of PCR product D with *Hph*I. β^0 Fs-6 (−A) and β^+ −87 (C-G) were detected from PCR product A digested with *Dde*I and *Avr*II, respectively. β^+ IVS-II nt 745 (C-G) was studied by digesting PCR product E with *Rsa*I.

DISCUSSION

Although there have been some reports about hemoglobinopathies, the real incidence of β-Thal in Argentina has not yet been determined [16,17]. Our institution is one of the largest referral centers for pediatric patients. Since 1991, 1,146 individuals have been studied to characterize their underlying erythrocyte abnormalities, and β-Thal syndromes comprised 19.9% of the diagnoses made.

We analyzed the spectrum of β-Thal genes in a group of Argentine patients, an ethnic group that had not been previously investigated at the molecular level. Since the majority of our patients with β-Thal are descendants of Italian or Spanish immigrants, we have studied the eight most frequent mutations in the Mediterranean countries. These mutations represent 95% of the affected genes in Argentina, and the four more common changes (C-39, IVS-I nt 110, IVS-I nt 1, and IVS-I nt 6) account for 84.7% of the β-Thal alleles in our population. As expected, all patients except 5 with β-Thal major were compound heterozygous; 2 of the homozygous patients had an IVS-I nt 110/IVS-I nt 110 genotype, and the other 3 had a C-39/C-39 genotype. This observation is not surprising since consanguinity is rare, the spectrum of alleles is quite wide, and the alleles involved were the most frequent. Interestingly, the allelic distribution was remarkably different between β-Thal major and β-Thal minor patients. In β-Thal major patients, β^+ and β^0 alleles were found in 53% and 40%, respectively. In contrast, in β-Thal minor patients β^+ alleles represented only 12%, while β^0 alleles contributed in 84% (χ^2 test, $P < 0.0035$). The mild phenotype present in β-Thal minor patients with a β^+ mutation may be responsible for the lack of proper diagnosis in our country, where β-Thal is not as common as in the Mediterranean area.

Comparison of our data with Mediterranean populations shows a close similarity between the allele distribution in Argentina and in Italy (Table III). Indeed, 87% of the β-Thal patients studied had an Italian origin, either in one or both parents.

The first flow of immigrants from Spain, at the beginning of the sixteenth century, established the urban settlements. So initially, the Argentine population grew on the basis of Spanish people, their descendants, aborigines, and mestizos. The Italian influence, on the other hand, is more recent. Not only was the Italian community in Argentina one of the first in the New World, but its global and quantitative evolution was different from other Italian communities in America as well. Its major significance was due to the large flow of immigrants that for more than five decades implied almost always a positive immigration balance [16,22]. In fact, the Italians represented practically the first massive European immigration to Argentina.

A recent report from Brazil describes the spectrum of mutations in that population [21]. The alleles found were very similar to the Italian ones but the distribution was very limited; only two mutations account for 84.3% of the affected alleles. These findings were to be expected based on the origin of Italian immigrants in Brazil, where most came from northern Italy and to a lesser extent from the southern regions.

Our data, instead, demonstrate a wider distribution, even though the most frequent mutations are the same. This could be explained by the number of Italian regions which contributed to the flow of immigrants. Before 1900 most of the Italians who arrived in Argentina were from Northern regions, but afterwards, they were outnumbered by people from the south, where β-Thal reaches its highest incidence [16]. The little differences observed between the Italian frequencies and the ones described here (Table III) might represent the influence of other immigrants, especially from Spain [18].

According to the frequency and distribution of the β-Thal alleles observed in the population under study, we can conclude that β-Thal in Argentina had mainly an Italian origin.

TABLE III. β-Thalassemia Mutations in Different Mediterranean and American populations*

	Nonsense codon 39	IVS-I nt 110	IVS-I nt 1	IVS-I nt 6	IVS-II nt 1	IVS-II nt 745	−87	Frameshift 6	Hb Lepore	Unidentified and others	Total
Italy[14]	40.1	23.0	10.2	9.9	3.9	5.0	1.4	1.2	1.2	9.3	100
Spain[18]	64.0	8.5	3.5	15.5				5.0		3.5	100
Tunisia[19]	19.0	7.5	1.5	10.5		7.5		16.0		38.0[a]	100
Turkey[20]	3.5	42	2.5	17.5	9.7	2.7	1.2	0.6		16.0[a]	100
Brazil[21]	64.3	20.0	7.1	5.7						2.9	100
Argentina	47.0	22.4	9.4	5.9	3.5	2.3	2.3	1.2	1.2	4.7	100

*Numbers refer to percentage of each allele.
[a]These values are due to local alleles.

This study will enable us to design a proper approach to genetic counseling and/or prenatal diagnosis for couples at risk.

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